Comparison of 4 laboratory methods for detection of *Helicobacter pylori*

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ABSTRACT

الأهداف: مقارنة استخدام الاختبارات الأربعة الشائعة في تشخيص الإصابة بعصيات هيلوكوباكتير (جرثومة المعدة) في المملكة العربية السعودية مع عسر الهضم.

الطريقة: أدرج في هذه الدراسة المرضى الذين قدموا إلى عيادة الجهاز الهضمي بمستشفى عسير المركزي بمدينة أبها والذين يعانون من سوء الهضم، في الفترة مابين أكتوبر 2005م ومايو 2006م. تم استبعاد المرضى الذين تلقوا العلاج المضاد للهيلوكوباكتير أو مثبطات مضخة البروتين خلال 30 يوماً أو الذين أجري لهم فحص بالمنظار وكشفت نتيجته وجود سرطان أو ارتداد معدي مريئي. تم فحص عينات البراز من أجل مستضد البراز لعصيات هيلوكوباكتير (HpSA) باستعمال تقنية الإنزيم المناعي من 2–7 أيام قبل الفحص بالمنظار. عند الفحص بمنظار البطن تم أخذ عينات من الغشاء المخاطي لغار المعدة لإجراء اختبار (CLO) والاختبار النسيجي والزراعة.

النتيجة: كان هنالك 72 ذكراً و43 أنثى تراوح العمر ما بين 18–75 عاماً، ومتوسط العمر 15.68±40.09، تبين وجود إصابة موجبة لعينة 79 مريضاً من بين 115 (68.7%) بعصيات هيلوكوباكتير بواسطة فحص الزراعة. توافق فحص الزراعة وفحص الأنسجة في 112 حالة (97.4%)، إلا أنهما اختلفا في 3 حالات (2.6%). النسبة المئوية للحساسية والمحدودية في تحليل الأنسجة كان 97.5 و 97.2، وفي HpSAg كانت 91.9 و98.6، وفي CLO كانت 79.7 و97.2 إختباراً ضد الثقافة.

خاتمة: إن فحص الزراعة وفحص الأنسجة وفحص (HpSA) جميعاً نتائج مقارنة وليس هنالك حاجة لاستخدام جميع الفحوصات الثلاثة في نفس الوقت من أجل تشخيص الإصابة بعصيات هيلوكوباكتير (جرثومة المعدة). فحص (CLO) أقل حساسية وقيمة التنبؤ السلبية لديه منخفضة.

Objective: To compare the usefulness of 4 commonly used tests in the diagnosis of *Helicobacter pylori (H. pylori)* infection in Saudi patients with dyspepsia.

Methods. Patients presenting with dyspepsia at the gastroenterology clinic of Aseer Central Hospital, Abha, Kingdom of Saudi Arabia between October 2005 to May 2006, who consented to participate in the study were enrolled. Patients who received anti-*Helicobacter* treatment or proton pump inhibitors within 30 days, or in whom endoscopy revealed cancer or gastro-esophageal reflux, were excluded from the study. Stool sample for *H. pylori* stool antigen (*Hp*SA) were tested using the enzyme immunoassay technique 2-7 days before endoscopy. At endoscopy, gastric antrum mucosal biopsies were taken for campylobacter-like organism (CLO) test, histology and culture.

Results. There were 72 males and 43 females, age range from 18-75 years, mean age 40.09±15.68. Seventy-nine patients out of the 115 (68.7%) samples were positive for *H. pylori*, by culture. Culture and histology agreed in 112 cases (97.4%) and disagreed in 3 cases (2.6%). The sensitivities and specificities (%) of histology were 97.5 and 97.2, of HpSAg were 91.9 and 98.6, and of CLO were 79.7 and 97.2 tests against culture.

Conclusion. Culture, histology, and *H. pylori* stool antigen tests all have comparable results, and there is no need to use all 3 at the same time, for the diagnosis of *H. pylori* infection. The CLO test is less sensitive, and of low negative predictive value.

Saudi Med J 2008; Vol. 29 (4): 530-532

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Received 25th June 2007. Accepted 23rd February 2008.

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Helicobacter pylori (*H. pylori*) a global human pathogen, is a major cause of gastritis and gastritis-related diseases and a precursor to the development of gastric carcinoma.[1-3] Multiple diagnostic methods are available for the detection of *H. pylori* infection, and these are put into 2 categories: invasive and non-invasive. The invasive tests include culture, histology, campylobacter-

like organism (CLO) test and polymerase-chain reaction (PCR). The non-invasive tests include serology, urea breath test (UBT), and stool antigen test. At present, no single test has been made the gold standard, although culture has a lot of advantages, and is irrefutable proof of *H. pylori* presence. In addition, it allows us to type the different strains, to test isolates for sensitivity to antimicrobial agents, to assay them for virulence factors, and to produce specific serological reagents.[4-6] Culture can be performed on biopsy specimens, or on gastric brush cytology specimens. The drawback of culture, however, include the choice of transport techniques since the organism is quite sensitive to atmospheric conditions, and the fact that this technique is not routinely available in several laboratories. Like in CLO-test, the organism is patchily distributed on the gastric mucosa, and a single biopsy specimen may not detect the bacterium. For these reasons, detection of *H. pylori* in culture ranges between 60-95%.[7,8] The objective of the present study was to compare 4 diagnostic methods for *H. pylori* (the CLO test, *Hp*SA test, culture, and histology) for the purpose of adopting a reliable, and reproducible technique for routine diagnosis of *H. pylori* infection.

Methods. Adult patients presenting with dyspepsia at the gastroenterology clinic of Aseer Central Hospital, Abha, Kingdom of Saudi Arabia between October 2005 to May 2006 who consented to participate in the study, were enrolled. Patients who received anti-*Helicobacter* treatment or proton pump inhibitors within 30 days, or in whom endoscopy revealed cancer or gastro-esophageal reflux, were excluded from the study. The patients were asked to present a stool sample at least 2 days to one week before endoscopy. The study was carried out between September 2005 to December 2006. The study was approved by the local ethical committee. At endoscopy, gastric antrum mucosal biopsy specimens were taken for CLO test (Ballard Medical Products Draper, Utah, USA), histology (Giemsa stain), and culture. The stool samples were sent to the Microbiology Department where they were frozen at -20^{0}C until tested. *Helicobacter pylori* antigens in stool were assessed by an enzyme immunoassay (Premier HpSA, Meridian Diagnostics, Inc. Cincinnati, OH, USA). The enzyme immunoassay was carried out according to the manufacturer's guidelines, and the results were read in spectrophotometer at 450 nm and 630 nm wavelengths. The equivocal results were repeated, and if the results were the same, they were regarded as negative. The absorbance of ≥0.120 was taken as a positive result. Biopsy samples for culture were stored in brucella broth with 20% glycerol at 4-8^{o}C, and picked up within 24 hours. They were inoculated on to brain-heart infusion agar plus 7% sheep blood to which was added vancomycin 10 mg/L, amphotericin B 10 mg/L, and trimethoprim 5 mg/L. The plates were incubated in moisture-enhanced microaerophilic atmosphere, using the Campypak sachet (Oxoid Limited, Hants, UK) for 5-7 days. Suspected colonies were confirmed as *H. pylori* if they were gram-negative curved rods, oxidase, and urease tests positive.

Statistical analysis was performed using the SPSS 14 package. To calculate simple statistics, sensitivities, specificities, positive predictive values, and negative predictive values.

Results. There were 115 patients, 72 were males and 43 were females. Their age range was 18-75 years and the mean age was 40.09 years±15.68. **Table 1** shows the sensitivity, specificity, positive-predictive value, and negative-predictive value of histology, HpSA, and CLO-tests in relation to culture, which was taken as the gold standard. Culture and histology agreed in 112 out of 115 cases (97.4%), 77 of these were positive cases, while 35 were negative cases (**Table 2**), while culture and *Hp*SA test agreed in 109 out of 115 cases (94.8%),

Table 1 - Comparison of diagnostic tests for *Helicobacter pylori* with culture as gold standard.

Characteristics	Histology	*Hp*SA test %	CLO test
Sensitivity	97.5	91.9	79.7
Specificity	97.2	98.6	97.2
Positive predictive value	98.7	99.3	98.4
Negative predictive value	94.6	89.4	68.6

*Hp*SA - *Helicobacter pylori* stool antigen,
CLO - campylobacter-like organism

Table 2 – Culture versus histology, *Hp*SA, and CLO test.

Test	Culture		
	Positive	Negative	Total
Histology			
Positive	77	1	**78**
Negative	2	35	**37**
Total	**79**	**36**	**115**
HpSA			
Positive	73	0	**73**
Negative	6	36	**42**
Total	**79**	**36**	**115**
CLO			
Positive	63	1	**64**
Negative	16	35	**51**
Total	**79**	**36**	**115**

*Hp*SA – *Helicobacter pylori* stool antigen,
CLO - campylobacter-like organism

73 of these were positive cases, while 36 were negative cases. The corresponding agreement for culture and CLO test was 98 out of 115 cases (85.2%), 63 of these were positive cases, while 35 were negative cases.

Discussion. A number of studies reporting on the tests for the diagnosis of *H. pylori* have been previously published in the Kingdom of Saudi Arabia[2,8] including this center,[9] however, not on culture technique. Other studies[1,3-7] had compared histology, CLO test, and *Hp*SA test with sensitivities, and specifics ranging between 80-100%. Histology was taken as the gold standard in the above reports. In our study, using culture as gold standard, the results of the CLO test were inferior to the other tests being less sensitive, and having a low negative predictive value. It is possible that some of our patients took anti-*Helicobacter* drugs against our instructions.

We used Brucella broth with 20% glycerol as transport medium and brain heart infusion agar supplemented with 7% sheep blood, and 3 antibiotics as culture medium.[10-11] These systems have worked well with us, and we recommend its use. Other researchers had used horse serum[4,7] however, this is more difficult to obtain, especially in the developing countries where sheep is more accessible, and cheaper. Speed and accuracy are taking the center stage in the diagnosis, and management of *H. pylori* infection. The use of PCR and UBT[11] offer great promise in this regard. These 4 tests have proved reliable, and reproducible in our hands, and any one or combination of them is recommended for the diagnosis of *H. pylori* infection, and the CLO test proved to be less sensitive compared to others. However, the global emergence of resistant bacteria, including *H. pylori*, makes the adoption of culture both pertinent, and urgent. More laboratories should therefore, employ this test in the Kingdom of Saudi Arabia as the gold standard, although cost wise, *Hp*SA test is the cheapest, reliable, and non-invasive.

Acknowledgment. *We are grateful to Prof. Ahmed Mahfouz of the Department of Community Medicine, College of Medicine, King Khalid University, Abha for the statistical analysis.*

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